GENE THERAPEUTICS

Methods and Applications of Direct Gene Transfer

Jon A. Wolff
Editor

Foreword by James F. Crow

92 Illustrations with some color art

Birkhäuser
Boston • Basel • Berlin

A HISTORY OF GENE TRANSFER AND THERAPY

Jon A. Wolff and Joshua Lederberg

INTRODUCTION

"Gene therapy" is generally thought of as a very new concept, but the notion that genes could be manipulated to treat human disease actually goes back several decades. Many of the pioneers of modern genetics realized that their discoveries eventually could lead to medical applications. Some even proposed gene transfer approaches decades ago that are still being explored today.

Perhaps the reason this history is little known is that progress in the science of gene therapy and transfer has been so slow in coming. However, the pace has accelerated in recent years, and many researchers are now embarking on clinical trials of very promising gene therapies. At this point, it seems useful to review the evolution of gene therapy's theoretical underpinnings and thereby gain insights into its medical, scientific, social, ethical, and economic implications. This historical perspective may be particularly useful for evaluation of the "prior art" in patent applications.

Several reviews have discussed some of the more recent advances in gene therapy (Miller, 1992; Friedmann, 1989) and in nonviral gene transfer methods (Felgner, 1990). One of the early proponents for gene therapy has recently reviewed its history (Friedmann, 1992).

GENETIC ENGINEERING

Since ancient times humans have bred animals and plants for specific purposes. We see the fruits of their labors when we eat corn, train a dog, race a horse, or smell a rose. Such selective breeding practices were the forerunners of the modern science of genetics, although the term "genetics" has come into use only relatively recently.

The "International Conference of Hybridization and on the Cross-Breeding of Varieties," held in 1899 in London, later came to be known as the "First International Congress of Genetics" (Crow, 1992). William Bateson suggested the use of the term "genetics" at the Third Congress in 1906. In the interim, H. de Vries and others had rediscovered Mendel's work, which originated the concept of the gene as a unit of hereditary.

Gene Therapeutics: Methods and Applications of Direct Gene Transfer
Jon A. Wolff, Editor • ©1994 *Birkhäuser Boston*

One of the first uses of the term "genetic engineering" was in a paper of that le presented at the "Sixth International Congress of Genetics" held in 1932 in naca, New York (Crow, 1992). The term was used to mean "the application of netic principles to animal and plant breeding." Nonetheless, Marxist commen-tors used the term "genetic engineering" for "eugenics" as contrast to the "social gineering" of the USSR (Lederberg, 1973).

HE PHARMACEUTICAL TRADITION

ne term "gene therapy," coined in part to distinguish it from the somewhat rwellian connotations of the term "human genetic engineering," can be defined "the application of genetic principles to the treatment of human disease." Un-er this broad definition, the highly successful screening programs for henylketonuria and Tay-Sachs disease could be included. Even many conven-onal medical and surgical therapeutic approaches, such as liver transplantation, ight fall under this definition because they were founded on an understanding of eir genetics and biochemistry, although they do not directly utilize genetic prin-ples.

More specifically, however, the term "gene therapy" combines the concepts pharmacotherapeutics with genetic principles. It implies the use of a substance treat the disease state.

In 1878, Langley proposed the concept of the "receptor substance," now known mply as the "receptor" (Goodman, 1975). The hypothesis that interactions be-veen a drug and its receptor are governed by the law of mass action was further eveloped by A.J. Clark in the 1920's. The molecular understanding of protein nction and enzyme action led to development of rational drug design based on rgeting the receptor, or active site (Perutz, 1992). Anti-sense and ribozyme gene erapies represent an extension of this concept.

Gene therapy approaches that involve gene addition or gene modification are onceptually similar to protein replacement therapies, such as those for diabetes ellitus and the hemophilias, in that a natural macromolecule of the human body administered. Transient gene therapy approaches such as the use of artificial RNA (Wolff, 1990) would also be similar to these protein replacement therapies. owever, nontransient gene therapy approaches such as retroviral vectors differ in at the administered gene may be permanently incorporated into the cells' chro-osomes. In this sense, gene therapy also resembles surgery, in which a tissue or rgan is permanently modified.

HE GENE AS A PHYSICAL ENTITY

very, MacLeod, and McCarthy first showed that a gene could be transferred ithin nucleic acids (Avery, 1944). Their 1944 article begins with a sentence per-nent to gene therapy:

> "Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters."

The ability for viruses to transmit genes was first demonstrated in Salmonella (Zinder and Lederberg, 1952). The idea that viral genomes could become a permanent part of cell genomes (i.e., prophages) was first discovered with bacteriophages (Lwoff, 1972; Lederberg, 1956) and then extended to animal viruses. For example, the ability of the Rous Sarcoma Virus to transform cells in culture, which in turn could produce new virus, led to the idea that the viral genes were responsible for the cells' transformation. Further studies of RSV infections in vitro led to the proviral hypothesis (Temin, 1976; Temin, 1971). Other studies demonstrated integration of SV40 viral DNA in SV40-transformed cells (Sambrook, 1968).

Watson and Crick's discovery of the structure of DNA and its implication concerning DNA's function had a revolutionary effect on biology (Watson, 1954). The history of the subsequent discoveries of mRNA and the formulation of the "central dogma" (genetic information flows from DNA to RNA to protein) is described in H. Judson's classic book entitled, "The Eighth Day of Creation" (Judson, 1979).

EARLY SPECULATIONS ON GENE THERAPY

Several key aspects of gene therapy were covered in a talk that Tatum gave in New York on May, 1966 (Tatum, 1966). One, that viruses could be used to transduce genes, is evident in his prediction that,

> "Finally, it can be anticipated that viruses will be effectively used for man's benefit, in theoretical studies in somatic-cell genetics and possibly in genetic therapy... We can even be somewhat optimistic on the long-range possibility of therapy by the isolation or design, synthesis, and introduction of new genes into defective cells of particular organs."

He also speculated that since the basis of cancer is altered genes, treatment could be achieved "by modification and regulation of gene activities, or by means of gene repair or replacement." Tatum also anticipated indirect or *ex vivo* approaches towards gene therapy:

> "Hence, it can be suggested that the first successful genetic engineering will be done with the patient's own cells, for example, liver cells, grown in culture. The desired new gene will be introduced, by directed mutation, from normal cells of another donor by transduction or by direct DNA transfer. The rare cell with the desired change will then be selected, grown into a mass culture, and reimplanted in the patient's liver. The efficiency of this process and its potentialities may be considerably improved by the synthesis of the desired gene according to the specifications of the genetic code and of the enzyme it determines, by in vitro enzymatic replication of this DNA, and by increasing the effectiveness of DNA uptake

and integration by the recipient cells, as we learn more about the factors and conditions affecting these processes."

e was extremely confident that gene therapy would be feasible, given our under-anding of the structure and function of genes.

One of us speculated about the possibility of gene therapy in an October 24, 962 letter to Stanfield Rogers:

"it will only be a matter of time, and perhaps not a long time, before polynucleotide sequences can be grafted by chemical procedures onto a virus DNA."

hese ideas were first published by Lederberg in a 1968 article that states,

"an attempt could then be made to transform liver cells of male offspring of haemophiliac ancestry by the introduction of carefully fractionated DNA carrying the normal alleles of the mutant haemophilia gene. This experiment would appear to be entirely analogous to the typical attempts at transforming bacterial forms. However, it is not clear whether one should regard this as a pure example of genetic engineering, since the practical outcome would probably be best achieved by influencing the nuclear consitution of somatic tissues rather than by direct tackling of the germ line. The precedent for this type of intervention would be the virus-mediated transduction of genetic characteristics that was also demonstrated in bacteria almost twenty years ago. The proposal, recently revived by Dr. S. Rogers, would require the discovery or artificial formation of cryptic viruses to which specified genetic information relevant to the cure of a genetic disease has been grafted. These viruses would then carry that information into the requisite cells of the host. Once the essential techniques for grafting segments of DNA from different sources onto that of a microbe have been perfected, experiments along these lines provide the most favourable opportunity to select those segments of DNA information which are needed. In this way it should not be extraordinarily difficult to obtain microbial DNA packets which are enriched with the gene, for example, for the synthesis of phenylalanine hydroxylase. One may of course argue that similar results could be achieved by the manipulation of tissue cells in culture as if they themselves were micro-organisms."

A. Kornberg's successful replication of DNA in a test tube was widely reorted in the popular press as the "creation of life in a test tube." It sparked an rticle by Lederberg on gene therapy that was published in the Washington Post in anuary 13, 1968.

W. Szybalski (Szybalski, 1991; Szybalski, 1992) who performed one of the arliest mammalian gene transfer experiments (see below), stated at a presentation o the Poultry Breeder's Round Table,

"When presenting our data at seminars and symposia in 1962-1964, we coined the terms 'gene surgery' and 'gene therapy' to stress the clinical potential of our work, but there was little interest in our results (except among poultry breeders),

probably because at that time prokaryotes, DNA synthesis, and the genetic code was the center of attention."

By the late 1960's and early 1970's, gene therapy became the subject of an increasing number of articles and meetings. Sinsheimer speculated on the prospect for "designed genetic change" of mankind (Sinsheimer, 1969). At an autumn, 1969 meeting (Aposhian, 1970), Aposhian proposed the use of pseudoviruses derived from the mouse virus, polyoma. He saw gene therapy arising from the pharmaceutical tradition.

"If one considers the purpose of a drug to be to restore the normal function of some particular process in the body, then DNA would be considered to be the ultimate drug."

In a 1970 *Science* article, B. Davis discussed human genetic engineering and explored the feasibility and ethics of several procedures, including somatic and germ cell alterations, cloning of humans, genetic modification of behavior, predetermination of sex, and selective reproduction (Davis, 1970). One of his major points was that "control of polygenic behavioral traits is much less likely than cure of monogenic diseases."

In 1971, a symposium on gene therapy was sponsored by the National Institute of Neurologic Disease and Stroke at the NIH and the Fogarty International Center (Publication, 1971). The first session entitled "Information transfer by mammalian viruses" included talks on recombinant SV40 viruses by D. Jackson and P. Berg and on RNA tumor viruses by H. Temin. The other sessions were entitled "Isolation of altered viruses with specific genes," "Information transfer by DNA," "Mammalian cellular systems," and "Immunologic and medical aspects." A discussion of gene therapy was offered in a 1972 *Science* article by Friedmann and Roblin (Friedmann, 1972). In 1976, gene therapy was the subject of another meeting sponsored by the New York Academy of Sciences (Morrow, 1976).

Obviously, then, the idea for gene therapy quickly occurred to several key researchers, once the basics of molecular genetics were established. However, despite these premonitions, Friedmann (Friedmann, 1990) observes that

"It has not always been quite so obvious as it is now that gene therapy is a rational and logically consistent approach to the treatment of some forms of human disease, from both the medical and scientific perspectives. Until fairly recently, the concept of gene therapy has been criticized by a sizable portion of the molecular biologic community as being remote and even improbable, possibly even unnecessary."

GENE TRANSFER INTO MAMMALIAN CELLS IN VITRO

Despite the unavailability of recombinant DNA and hybridization techniques, several studies attempted to demonstrate that mammalian cells were able to take up

NA, ist as bacteria do. Several studies in the late 1950's and early 1960's demstrad that cultured cells took up radioactive DNA (Sirotnak, 1959; Schimizu, 62; Gartler, 1960; Mathias, 1962; Kay, 1961; Hill, 1970; Gartler, 1959; reneund, 1961; Rabotti, 1963; Azrin, 1961). Some of these studies also rerteentry of the radioactive DNA into the nucleus of the cells.

Te study of viruses has motivated several early efforts for transferring genes o mmmalian cells. In the late 1950's and early 1960's, several studies demonatedhat naked, viral DNA or RNA can be infective when applied to cells. This is fit discovered with plants and tobacco mosaic virus in 1956 and then quickly tened to poliomyelitis, Semliki Forest encephalitis, influenza and several other uscin mammalian cells. These studies prompted a Science review article which scued the infectious disease implications of these studies (e.g., "their release om ifected tissues and resistance to antibodies may explain some anomalous ndions") (Herriott, 1961). The infectious entity presumed to be polynucleotides is ctained by phenol-extraction of the virus, was labile to nucleases, and was t nitralized by antibodies. For example, a phenol-extract of polio virus yields NAhat produces plaque-forming poliovirus when injected into embryonated gs Mountain, 1959) or when applied to monkey kidney tissue cultures (Dubes, 61)(Klingler, 1959) mouse embryo cells (Weil, 1961), or HeLa cells (Alexander, 58)

Another series of studies explored the effect of viral RNA concentration, soliocomposition, and temperature to cause infections of Mengo encephalomyelitis mouse fibroblasts (Colter, 1957; Colter, 1961). They found that hypertonic sae ad sucrose solutions increased the infectivity of the RNA. Another study also undhat exposure of HeLa cells to hypertonic solutions increased the number of aqus formed from polioviral RNA (Koch, 1960). In 1960, it was reported that olicdral RNA uptake was enhanced by high concentrations of magnesium sulte (M) (Holland, 1960). In 1961, Dubes and Klinger reported increased effienc of polioviral RNA plaque formation with the use of calcium depleted cells id 'oorly soluble substances" such as $CaHPO_4{\cdot}2H_2O$ and Cr_2O_3, Al_2O_3, $CaCO_3$, aSO_4, Fe_2O_3, MgF_2 (Dubes, 1961). A footnote reported that

> nfection is also facilitated by the fine cloudly precipitate, very probably a calum phosphate, formed when phosphate-buffered saline is made by mixing its gredients before sufficient dilution with water."

everal early studies reported increased uptake of cellular or viral polynucleid by complexing them with various proteins. Amos found that the uptake of diabelled *E. coli* RNA by cultured chick cells was enhanced by complexing it ithbrotamine (Amos, 1961). Other polycations such as streptomycin, spermine, id permidine did not increase uptake but were observed to protect the RNA onRNase and caused precipitates to form in some formulations (Amos, 1961). mul and Ludwig, in 1962, reported that calf thymus histone or protamine enand the infectivity of polioviral and Coxsackie B_3 RNA in HeLa cells but did

not protect the RNA from RNase digestion (Smull, 1962). Another study found that methylated albumin (a basic protein) protected DNA and RNA from nucleases and enhanced their uptake by HeLa cells (Cocito, 1962). Bensch and King's observations that L cells did not take up appreciable amounts of DNA but did phagocytose particles prompted them to incorporate DNA into particles (Bensch, 1961). Using the Feulgen stain, acridine organge, and radioactive DNA, they found that DNA complexed with charcoal or activated resin did not increase DNA's uptake. However, DNA incorporated into 0.5-50 μm-sized, gelatin particles entered the cytoplasm and nucleus of the L cells. Much later in 1975, Farber and co-workers reported that cultured Chinese hamster lung cells took up more radiolabelled genomic DNA when complexed with polyornithine than with DEAE-dextran, 125 mM $CaCl_2$, latex spheres, spermine, polylysine, and polyarginine (Farber, 1975).

Several studies in the early 1960's reported changes in cellular phenotype by the transfer of foreign genes. Kraus reported in *Nature* in 1961 that bone marrow cells in culture from a patient homozygous for sickle cell disease expressed the normal β-globin polypeptide (per electrophoresis) when the cells were exposed to DNA from normal bone marrow cells (Azrin, 1961). Weisburger reported in the *Proceedings of the National Academy of Science* that the transfer of ribonucleoprotein (resistant to DNase but sensitive to RNase and trypsin digestion) from a normal bone marrow or spleen caused the expression of normal hemoglobin in sickle cell bone marrow and reticulocytes as determined by electrophoresis, column chromatography and peptide digests (Weisberger, 1962). Also in the *Proceedings* in 1962, Kantoch and Bang reported that they were able to transfer genetic susceptibility to mouse hepatitis virus infection between macrophages of two different mouse strains (Kantoch, 1962). In their discussion, they reported that this transfer was blocked by incubating the extract with DNase. In 1962, French workers reported that they could modify the karyotype of chicken cells by exposing them to cow DNA (Frederic, 1962). Another study noted increased survival of irradiated, L cells if they were exposed to DNA from nonirradiated L cells (Djordjevic, 1962). Several negative results concerning DNA transfer were reported as well in the early 60's. Mathias and Fisher attempted to transfer amethopterin resistance in mouse leukemic cells without success (Mathias, 1962). The exposure of donor, bone marrow cells to naked and gelatin-complexed DNA from the host bone marow cells in isotonic and hypertonic solutions did not affect the success of bone marrow transplantation in mice (Floersheim, 1962). The premise was that transfer of histocompatibility genes would induce immunotolerance.

The development of cell lines containing defined enzymatic defects and selectable systems proved extremely useful in gene transfer studies and ushered in the modern era of gene transfer. W. and E. Szybalski developed HPRT-deficient cell lines and the HAT selection media. DNA isolated from HPRT+ cells was able to confer HAT-resistance to HPRT- cells. The DNA was transferred in a phosphate buffer containing spermine in order to bind the DNA and shield it from DNase activity (Szybalska, 1962). In Table I of their 1962 article, they showed a dose-dependent relationship between the amount of donor DNA and the number of

ansformants. Subsequent experiments indicated that the spermine contained 30% aCl_2 and that the spermine could be replaced by $CaCl_2$ when used with phos-ıate buffers. Precipitates were observed during these experiments. These later ‹periments with $CaCl_2$ were reported in Table 2 of their paper in Proceedings of ıe 12th Annual Session National Poultry Breeder's Roundtable (Szybalski, 1963) ı 1963:

> "It was noticed that only one of preparations of spermine was especially active in the transformation process. Addition of this spermine solution to the phosphate-buffered saline (PBS) resulted in clouding of the solution, both in the presence or absence of the transforming DNA and the cells. Since we found that the particular spermine preparation contained a high concentration of calcium, the precipitate was most probably the calcium phosphate."

Further chemical analysis indicated that the contaminant was in fact calcium Szybalski, 1992). This finding was not widely disseminated because of the spe-ıalized nature of the publication in which it were reported, but Szybalski's early ɔntribution to DNA-mediated gene transfer was recognized by Scangos and Ruddle ı a 1981 review (Scangos, 1981). Also, in 1962, Bradley, Roosa, and Law used 8-zaguanine selection to demonstrate the cellular uptake of naked, genomic DNA Bradley, 1962).

Despite these early observations concerning calcium phosphate precipitation, ıost workers used DEAE-dextran to transfer foreign DNA into mammalian cells s a result of a report by Vaheri and Pagano in 1965 that showed increased transfer f polio virus RNA with DEAE-dextran (Vaheri, 1965). This 1965 study com-ared the use of DEAE-dextran to that of hypertonic magnesium solutions. Burnett nd Harrington also used DEAE-dextran to transfer polyoma virus DNA but indi-ated that this was not successful with adenoviral DNA (Burnett, 1968). Subse-uently several other studies reported the successful use of DEAE-dextran. 1cCutchan and Pagano transferred SV40 DNA(McCutchan, 1968), Warden and 'horne transferred polyoma virus, (Warden, 1968), and Nicolson and McAllister ansferred adenovirus 1 (Nicolson, 1972). Hill and Hillova produced infectious .SV after non-infected cells were transfected using DEAE-dextran with DNA om Rous sarcoma virus infected cell (Hill, 1972).

It was not until the detailed study of Graham and Van Der Eb on calcium hosphate-mediated transfection that this technique became widely used and ac-epted (Graham, 1973). They systematically explored the use of calcium or mag-esium ions for transfection and determined that co-precipitates of DNA, calcium nd phosphate were necessary for efficient transfection. They also explored sev-ral parameters, such as pH (6.9-7.4), incubation times, confluency of cells (60-0%) and adenoviral and carrier DNA concentration, in a systematic fashion. ecause of this and the reproducible, 50- to 100-fold increase in efficiency over)EAE-dextran, this study had a major impact on the field and is widely cited as ıe primary source for the "calcium phosphate transfection" technique. After their

initial publication in 1973, they modified the procedure further in 1974 (Graham, 1974),

EARLY ATTEMPTS AT DIRECT GENE TRANSFER IN VIVO

Perhaps the earliest predecessor of a direct in vivo approach was the use of vaccines, which permanently modify the body's response to infection. Vaccination with attenuated viruses may be viewed as a form of gene therapy, especially since the viral genomes may persist long-term. The ease of administration, relative cheapness, and long-lasting effect of vaccines are ideal qualities to which proponents of direct gene therapy aspire.

Another idea for direct, in vivo therapy was the notion of treating bacterial infections by the injection of bacteriophages. While this therapeutic approach was discussed in Sinclair Lewis' novel Arrowsmith, there were several actual reports of its successful use in animals and humans (d'Herelle, 1926). The negative results of well-controlled studies (Boyd, 1944) and the advent of antibiotics stopped its further investigation. Interestingly, there have been some recent reports of its exploration in animals (Reynaud, 1992; Soothill, 1992).

Several early studies reported the direct transfer of polynucleotides into tissues in vivo and in situ. Rieke reported that periotoneal malignant and normal cells in the peritoneum took up radioactive DNA (Rieke, 1962). Rabotti reported the uptake of radioactive DNA into tumors in vivo (Rabotti, 1963), but the foreign DNA demonstrated no functional activity. Radioactive DNA injected intravenously or intraperitoneally in rodents were taken up by spleen and bone marrow cells (Hudnik-Plevnik, 1959; Hill, 1961). Other studies reported the uptake of labelled DNA by cells in mice in vivo.

J. Benoit et al. reported beginning in 1956 that Peking ducklings injected intraperitoneally with DNA extract from Khaki Campbell ducks exhibited characteristics of the Khaki Campbell ducks in terms of body and head size, and that these effects were passed onto their progeny (Benoit, 1960; Benoit, 1960). Much to the dismay of paté manufactures and Chinese chefs who were expecting a culinary breakthrough, these results in Ducks have never been reproduced.

The Benoit studies in ducks attracted enough attention to prompt several other investigators to attempt phenotypic modifcation by DNA transfer in other species. The injection of rat DNA from a pigmented rat into an albino rat did not produce any change in skin color (Perry, 1958). Two other studies also reported the inability to produce pigmentary studies in albino rodents by injection of DNA from a pigmented rodent (Bearn, 1961), (Holoubek, 1961). In addition, the intraperitoneal injection of DNA from a normal rat did not correct the hyperbilirubinemic state of the CNH strain (noted to be deficient in glucuronyl transferase) (Perry, 1958). In chickens, the injection of a "Tyrode" solution or DNA in the Tyrode solution into the blood stream of young chicken embyros did not cause any morphological changes but did cause teratogenic malformations (Martinovitch, 1962).

mice, another group observed that the intraperitoneal injection of DNA from reast cancers of agouti C_3H mice but not DNA from other organs caused cyto-gical changes in the livers of white mice (Leuchtenberger, 1958). Two different roups noted that injection of DNA from one mouse strain caused weak transplan-tion immunity against skin grafts but raised the possiblity that contaminants may ave been responsible for the effect (Haskova, 1958; Medawar, 1958).

In summary, the Benoit studies prompted many attempts to research DNA otake by vertebrate cells. However, the study made the entire field of gene trans-r into cells of higher organisms somewhat suspect (W. Szybalski, personal com-unication).

Other studies explored the ability of DNA to transfer the neoplastic state. In e early 1950's, it was reported that new tumors formed after injection of DNA om tumor cells into normal mouse tissues (Stasney, 1950), (Paschkis, 1955). ne-third of rats subcutaneously injected with lymphosarcoma chromatin were id to have developed lymphosarcomas or leukemia, while approximately one-ird of rats injected intrahepatically were said to have developed hepatomas. A bsequent report observed a similar phenomenon but concluded that it was the sult of contaminating cancer cells (Klein, 1952). A 1958 report observed that the peated, subcutaneous injections of herring-sperm DNA caused duodenal denocarcinoma in two mice but this was not repeatable with a different batch of NA (Meek, 1959). This report prompted a subsequent study that reported that peated, subcutaneous injections of herring-sperm DNA caused intestinal carci-omas in cichlids (Stolk, 1960). The injection of *Drosophila melanogaster* DNA as also noted to be mutagenic in *Drosophila* (Fahmy, 1961). Only many years ter was the phenotype for neoplastic transformation reliably transferred from ammalian DNA into cells in culture (Cooper, 1980; Shih, 1979). Nonetheless, veral studies in the late 1950's and early 1960's reported neoplastic transforma-on by viral polynucleotides in vivo just as there was a flurry of reports at this time oncerning the infectivity of viral polynucleotides. For example, phenol extracts SE polyoma virus were able to cause infections and tumors in hamsters DiMayorca, 1959). Also, phenol extracts of papillomatous tissue (Shope) of Cot-ntail rabbits, produced paillomas when injected into the skin of rabbits (Ito, 1960), to, 1961).

The development of plasmid expression vectors, reporter genes, and better in tu detection systems prompted more recent attempts at direct, in vivo gene trans-r. In 1983, large liposomes containing the rat preproinsulin gene within a plas-id were injected intravenously into rats (Nicolau, 1983). The injections caused a 30% decrease in blood glucose and a ~50% increase in blood insulin.

Another early study involved the injection of calcium phosphate-precipitated olyoma viral DNA into mouse liver and spleen along with hyaluronidase and ollagenase (Dubensky, 1984). The investigators reported the presence of the olyoma DNA in the tissues and inferred that the viral DNA had to replicate. imilar studies were also done with polyoma DNA and proteoliposomes (Mannino, 988).

Another study involved the intraperitoneal injection of calcium phosphate-precipitated plasmids containing chloramphenicol acetyltransferase (CAT), hepatitis B surface antigen, human growth hormone, or mouse preproinsulin genes (Benvenisty, 1986). The investigators observed some CAT activity, immunohistochemical staining for the hepatitis antigen, insulin RNA, and growth hormone RNA in livers injected with the respective plasmids.

FIRST ATTEMPTS AT HUMAN GENE THERAPY

In the late 1960s, S. Rogers injected the Shope papilloma virus into patients with arginase deficiency, based upon his studies that indicated that the virus contained an arginase gene. His initial observation was that rabbit skin tumors induced by the Shope papilloma virus contained high levels of arginase activity (Rogers, 1963; Rogers, 1959). Since he did not find any arginase activity in normal rabbit skin, he concluded that the virus carried an arginase gene. He also reported that the virus induced a virus-specific arginase in fibroblasts from a patient with arginase deficiency (Rogers, 1973). A biochemical assay demonstrated increased arginase activity, and an immunohistochemical stain with anti-sera specific against the virus-specific arginase distinguished the virus-induced arginase from native arginase. Administration of the virus to animals caused no harmful effect and reduced blood arginase levels. However, other workers found arginase activity in normal skin (Rothberg, 1961; Orth, 1967). These other workers also showed that rabbit liver arginase and Shope had similar kinetic and antigenic properties and that papillomas induced by a carcinogen also contained arginase. They concluded that the Shope virus either induces arginase expression or leads to the preferential growth of cells with higher arginase activity. The final outcome to this controversary was that three siblings with arginase deficiency were injected with the Shope virus, without any effect on their arginine levels (Terheggen, 1975).

In a 1980 *Nature* report, Cline reported that DNA from a highly methotrexate-resistant Swiss 3T6 cell line (containing many copies of the DHFR gene) was calcium phosphate transfected into mouse bone marrow cells (Cline, 1980). The donor bone marrow cells had a different karyotype to distinguish them from the recipient bone marrow cells. The recipient mice were irradiated and treated with methotrexate before being injected with the transfected cells. After transplantation, the recipient animals were reported to have an increased percentage of marrow cells with the donor karyotype and increased DHFR enzymatic activity. A similar study was published in *Science* which used recombinant DNA containing the herpes TK gene in the pBR322 plasmid vector (Mercola, 1980). On the basis of this experimental data they attempted to calcium phosphate transfect the β-globin gene into human bone marrow cells, which were then transplanted into patients with thalasemia. Their clinical trial was criticized for both scientific and procedural reasons, and this led to Cline's censure by the NIH and by his university (Wade, 1980; Wade, 1981; Wade, 1981). As a result, the NIH decided that all

cal practice. Rather, gene therapy is an entirely new branch of medicine, one that could potentially revolutionize the way we treat human disease.

REFERENCES

Alexander HE, Koch G, Mountain IM, Sprunt K, Van Damme O (1958): Infectivity of ribonucleic acid of poliovirus on HeLa cell monolayers. *Virology* 5:172-173

Amos H (1961): Protamine enhancement of RNA uptake by cultured chick cells. *Biochem Biophys Res Comm* 5:1-4

Anderson WF, Fletcher JC (1980): Gene therapy in human beings: when is it ethical to begin? *N Eng J Med* 303(22):1293-1297

Anderson WF, Killos L, Sanders-Haigh L, Kretschmer PJ, Diacumakos EG (1980): Replication and expression of thymidine kinase and human globin genes microinjected into mouse fibroblasts. *Proc Natl Acad Sci USA* 77(9):5399-5403

Aposhian HV (1970): The use of DNA for gene therapy—the need, experimental approach, and implications. *Perspectives in Biology and Medicine* 14:987-108

Aposhian HV, Qasba PK, Osterman JV, Waddell A (1972): Polyoma pseudovirions: an experimental model for the develpment of DNA for gene therapy. *Federation Proceedings* 31(4):1310-1325

Aposhian HV, Qasba PK, Osterman JV, Waddell A (1972): Polyoma pseudovirions: an experimental model for the develpment of DNA for gene therapy. *Federation Proceedings* 31(4):1310-1325

Avery OT, MacLeod CM, McCarty M (1944): Studies on the chemical nature of the substance inducing transformation of pneumococcal types. *Journal of Experimental Medicine* 79:137-158

Azrin NH (1961): Incorporation of heterologous deoxyribonucleic acid into mammalian cells. *Science* 133:381-383

Bacchetti S, Gaham FL (1977): Transfer of the gene for thymidine kinase to thymidine kinase-deficient human cells by prurified herpes simplex viral DNA. *Proc Natl Acad Sci USA* 74(4):1590-1594

Baltimore D (1970): Viral RNA-dependent DNA polymerase. *Nature* 226:1209-1211

Bearn JG, Kirby KS (1961): Failure of deoxyribonucleic acid to produce pigment changes in the albino rat. *Exp Cell Res* 17:547-549

Benoit J, Leroy P, Vendrely R, Vendrely C (1960): Experiments on pekin ducks treated with DNA from khaki campbell ducks. *Transaction of The New York Academy of Sciences* 22: 494-503

Benoit J, Leroy P,Vendrely R, Vendrely C (1960): Modifications de caracteres raciaux du canard pekin par l'acide desoxyribonucleique de canard khaki cambell et leur transmission a la descendance. *Biochemical Pharmacology* 4:181-194

Bensch KG, King DW (1961): Incorporation of heterologous deoxyribonucleic acid into mammalian cells. *Science* 133:381-382

Benvenisty N, Reshef L (1986): Direct introduction of genes into rats and expression of the genes. *Proc Natl Acad Sci USA* 83:9551-9555

Borenfreund E, Bendich A (1961): A study of the penetration of mammalian cells by deoxyrobonucleic acids. *The Journal of Biophysical and Biochemical Cytology* 9:81-91

Boyd JSK, Portnoy B (1944): Bacteriophage therapy in bacillary dysentery. *Trans R Soc Trop Med Hyg* 37:243-262

Bradley TR, Roosa RA, Law LW (1962): DNA transformation studies with mammalian cells in culture. *Journal of cellular and comparative physiology* 60:127-138

Burnett JP, Harrington JA (1968): Infectivity associated with Simian Adenovirus Type SA7 DNA. *Nature* 220:1245

Cline MJ, Stang H, Mercola K, Morse L, Ruprecht R, Browne J, Salser W (1980): Gene transfer in intact animals. *Nature* 284:422-5

Cocito C, Prinzie A, De Somer P (1962): Uptake by mammalian cells of nucleic acids combined with a basic protein. *Experientia* 18:218-220

Cold Spring Harbor Laboratory (1983): *Gene Therapy: Fact and Fiction.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory

Colter JS, Bird HH, Moyer AW, Brown RA (1957): Infectivity of ribonucleic acid isolated from virus-infected tissues. *Virology* 4:522-532

Colter JS, Ellem KAO (1961): Interaction of viral nucleic acids with mammalian cells. *Federation Proceedings* 20:650-655

Cooper GM, Okenquist S, Silverman L (1980): Transforming activity of DNA of chemically transformed and normal cells. *Nature* 284:418-421

Crow JF (1992): Anecdotal, Historical and Critical Commentaries on Genetics. *Genetics* 131:761-768

Cushman DW, Ondetti MA (1991): History of the design of captopril and related inhibitors of angiotensin converting enzyme. *Hypertension* 17(4):589-592

d'Herelle F (1926): *The bacteriophage and its behavior.* (Smith GH, Trans.). Paris: Balliere

Davis BD (1970): Prospects for Genetic Intervention in Man. *Science* q170:1279-1283

DiMayorca GA, Eddy BE, Stewart SE, Hunter WS, Friend C, Bendich A (1959): Isolation of infectious deoxyribonucleic acid from SE polyoma-infected tissue cultures. *Proc Natl Acad Sci USA* 45:1805-1808

Djordjevic O, Kostic L, Kanazir D (1962): Recovery of ultra-violet-irradiated L strain cells by means of highly polymerized deoxyribonucleic acid. *Nature* 195:614-615

Doehmer J, Barinaga M, Vale W, Rosenfeld MG, Verma IM, Evans RM (1982): Introduction of rat growth hormone gene into mouse fibroblasts via a retorviral DNA vector: Expression and regulation. *Proc Natl Acad Sci USA* 79:2268-2272

Dubensky TW, Campbell BA, Villarreal LP (1984): Direct transfection of viral

and plasmid DNA into the liver or spleen of mice. *Proc Natl Acad Sci USA* 81:7529-7533

Dubes GR, Klingler EA (1961): Facilitation of Infection of Monkey Cells with Poliovirus "Ribonucleic Acid". *Science* 133:99-133

Fahmy OG, Fahmy MJ (1961): Induction of mutations by deoxyribonucleic acid in Drosophila melanogaster. *Nature* 191:776-779

Farber FE, Melnick JL, Butel JS (1975): Optimal conditions for uptake of exogenous DNA by chinese hamster lung cells deficient in hypoxanthine-guanosine phosphoribosyltransferase. *Biochim Biophys Acta* 390:298-311

Felgner PL (1990): Particulate systems and polymers for in vitro and in vivo delivery of polynucleotides. *Advanced Drug Delivery Reviews* 5:163-187

Floersheim GL (1962): System for the recognition of genetic transformation in haemopoietic cells. *Nature* 193:1266-1268

Fournier REK, Ruddle FH (1977): Microcell-mediated transfer of murine chromosomes into mouse, Chinese hamster, and human somatic cells. *Proc Natl Acad Sci USA* 74(1):319-323

Frederic J, Corin-Frederic J (1962): Modifications des chromosomes et du caryotype dans des cellules de Poulet cultivees in vitro en presence d'acides desoxyribonucleiques de veau. *C R Soc Biol* 156:742-745

Friedmann T (1971): In vitro reassembly of shell-like particle from disrupted polyoma virus. *Proc Natl Acad Sci USA* 68(10):2574-2578

Friedmann T (1976): The future for gene therapy—a reevaluation. *Annals New York Academy of Science* 265:141-152

Friedmann T (1989): Progress toward human gene therapy. *Science* 244:1275-1281

Friedmann T (1990): The Evolving Concept of Gene Therapy. *Human Gene Therapy* 1:175-181

Friedmann T (1992): A brief history of gene therapy. *Nature genetics* 2(october):93-98

Friedmann T, Roblin R (1972): Gene therapy for human genetic disease? *Science* 175(4025):949-955

Gartler SM (1959): Cellular uptake of deoxyribonucleic acid by human tissue culture cells. *Nature* 184:1505-1506

Gartler SM (1960): Demonstration of cellular uptake of polymerized DNA in mammalian cell cultures. *Biochem Biophys Res Comm* 3:127-131

Goff SP, Berg P (1976): Construction of hybrid viruses containing SV40 and gamma phage DNa segments and their propagation in cultured monkey cells. *Cell* 9:695-705

Goodman LS, Gilman A (Ed.). (1975): *The Pharmacologic Basis of Therapeutics*. New York: Macmillan Publishing Co

Graessmann M, Graessmann A (1976): "Early" simian-virus-40-specific RNA contains information for tumor antigen formation and chromatin replication. *Proc Natl Acad Sci USA* 73:366-370

Graf BH, Urlaub G, Chasin LA (1979): Transformation of the gene for hypoxanthine phophoribosyltransferase. *Somatic Cell Genetics* 5(6):1031-1044

Graham FL, Van Der Eb AJ (1973): A New technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52:456-467

Graham FL, Van der Eb AJ, Heijneker HL (1974): Size and location of the transforming region in human adenovirus type 5 DNA. *Nature* 251:687-90

Green MR, Treisman R, Maniatis T (1983): Transcriptional Activation of Cloned Human Beta-Globin Genes by Viral Immediate-Early Gene Products. *Cell* 35:137-148

Hamer DH, Davoli D, Thomas CA, Fareed GC (1977): Simian virus 40 carrying an Escherichia coli Suppressor Gene. *Journal of Molecular Biology* 112:155-182

Hamer DH, Leder P (1979): Expression of the chromosomal mouse β^{maj}-globin gene cloned in SV40. *Nature* 281:35-40

Hamer DH, Smith KD, Boyer SH, Leder P (1979): SV 40 Recombinants Carrying Rabbit bet-Globin Gene Coding Sequences. *Cell* 17:725-735

Haskova V, Hrubesova M (1958): Part played by deoxyribonucleic acid in transplantation immunity. *Nature* 182:61-62

Herriott RM (1961): Infectious nucleic acids, a new dimension in virology. *Science* 134:256-260

Hershey AD, Burgi E (1965): Complementary structure of interacting sites at the ends of lambda DNA molecules. *Proc Natl Acad Sci USA* 53:325-328

Hill M (1961): Uptake of deoxyribonucleic acid (DNA): a special property of the cell nucleus. *Nature* 189:916-917

Hill M, Hillova J (1972): Virus Recovery in chicken Cells tested with Rous Sarcoma Cell DNA. *Nature New Biology* 237:35-39

Hill M, Huppert J (1970): Fate of exogenous mouse DNA in chicken fibroblasts in vitro non-conservative preservation. *Biochimica and Biophysica Acta* 213:26-35

Hill M, Jakubickova J (1962): Intercellular passage of DNA as revealed in bone marrow autoradiographs. *Exp Cell Res* 26:541-551

Holland JJ, Hoyer BH, McLaren LC, Syverton JT (1960): Enteroviral ribonucleic acid. I—Recovery from virus and assimilation by cells. *J Exp Med* 112:821-864

Holoubek V, Hnilica L (1961): The failure to produce somatic and immunologic changes in inbred AKR mice by specific deoxyribonocleic acid. *Can J Biochem Physio* 39:1478-1479

Hudnik-Plevnik T, Glisin VR, SImic MM (1959): Fate of the highly polymerized spleen deoxyribonucleic labelled with Phosphorus-32 injected intraperitoneally into rats. *Nature* 184:1818-1819

Ito Y (1960): A tumor-producing factor extracted by phenol from papillomatous tissue (Shope) of Cottontail Rabbits. *Virology* 12:596-601

Ito Y (1961): Heat-resistance of the tumorigenic nucleic acids of Shope papillomatosis. *Proc Natl Acad Sci USA* 47:1897-1900

Jackson DA, Symon RH, Berg P (1972): Biochemical method for inserting new

genetic information into DNA of Simian Virus 40: Circular SV40 DNA molecules containing lambda phage genes and the galactose peron of Escherichia coli. *Proc Natl Acad Sci USA* 69(10):2904-2909

Judson HF (1979): *The Eighth Day of Creation: The makers of the revolution in biology*. New York: Simon and Schuster.

Kaneda Y, Iwai K, Uchida T (1989): Increased Expressio of DNA cointroduced with nuclear Protein in adult rat liver. *Science* 243:375-377

Kantoch M, Bang FB (1962): Conversion of genetic resistance of mammalian cells to susceptibility to a virus infection. *Proc Natl Acad Sci USA* 48:1553-1559

Kao F-T, Puck TT (1968): Genetics of somatic mammalian cells, VII. Induction and isolation of nutritional mutants in chinese hamster cells. *Proc Natl Acad Sci USA* 60:1275-1281

Kay ERM (1961): Incorporation of Deoxyribonucleic Acid by mammalian cells in vitro. *Nature* 191:387-388

Keating A, Toneguzzo F (1990). Gene tranfer by electroporation: a model for gene therapy. In S. Gross,A. P. Gee, D. A. Worthington-White (Eds.), *Bone Marrow Purging and Processing*. (pp. 491-498). New Yord: Wiley-Liss

Klein G (1952): The nature of mammalian lymphosarcoma transmission by isolated chromatin fractions. *Cancer Research* 12:589-590

Klingler E, Chapin M, Dubes GR (1959): Relationship between inactivation of poliovirus by phenol and appearance of ribonuclease-labile infectivity. *Proc Soc Exp Biol Med* 101:829-832

Kobernick SD, Toovey EW, Webster DR (1952): Effects of the Application of Carcinogens to exposed gastric mucosa in the rat. *Cancer Research* 12:591-593

Koch G, Koenig S, Alexander H (1960): Quantitative studies on the infectivity of ribonucleic acid from partially purified and highly purified poliovirus preparations. *Virology* 10:329-343

Kraus L (1961): Formation of different haemoglobins in tissue culture of human bone marrow treated with human deoxyribonucleic acid. *Nature* 192:1055-1057

Lederberg J (1956): Genetic transduction. *American Scientist* 44:264-280

Lederberg J (1973): The genetics of human nature. *Social Res* 40:375-406

Leuchtenberger C, Leuchtenberger R, Uyeki E (1958): Cytological and cytochemical changes in livers of white mice following intraperitoneal injections of DNA preparations from breast cancers of Agouti C3H mice. *Proc Natl Acad Sci USA* 44:700-705

Levine F, Friedmann T (1991): Gene therapy techniques. *Current Opinion in Biotechnology* 2:840-844

Lewis W, Srinivasan PR, Stokoe N, Siminovitch L (1980): Parameters governing the transfer of the genes for thymidine kinase and dihydrofolate reductase into mouse cells using metaphase chromosomes or DNA. *Somatic Cell Genetics* 6(3):333-347

Linnane AW, Lamb AJ, Christodoulou C, Lukins HB (1967): The biogenesis of

mitochondria, VI. Biochemical basis of the resistance of saccharomyces cerevisiae toward antibiotics which specifically inhibit mitochondrial protein synthesis. *Proc Natl Acad Sci USA* 59:1288-1293

Lwoff A (1972). Interaction among virus, cell, and organism:Nobel lecture, December 11, 1965. In *Nobel Lectures in Physiology or Medicine, 1963-1970* (pp. 174-185). New York: Elsevier

Maitland NJ, McDougall JK (1977): Biochemical transformation of Mouse Cells by Fragments of Herpes Simplex Virus DNA. *Cell* 11:233-241

Mandel M, Higa A (1970): Calcium-dependent bacteriophage DNA Infection. *Journal of Molecular Biology* 53:159-162

Maniatis T, Kee SG, Efstratiadis A, Kafatos FC (1976): Amplification and characterization of a Beta-Globin Gene Synthesized in vitro. *Cell* 8:163-182

Mannino RJ, Gould-Fogerite S (1988): Liposome Meidated Gene transfer. *BioTechniques* 6(7):682-90

Martinovitch PN, Kanazir DT, Knezevitch ZA, Simitch MM (1962): Teratological changes in the offspring of chicken embryos treated with tyrode or with tyrode plus DNA. *J Embryol Exp Morph* 10:167-177

Mathias AP, Fischer GA (1962): Transformation experiments with murine lymphoblastic cells (L5178Y) grown in culture. *Biochemical Pharm* 11:69-78

McCutchan JH, Pagano JS (1968): Enhancement of the infectivity of Simian Virus 40 Deoxyribonucleic Acid with Diethylaminoethyl-Dextran. *Journal of the National Cancer Institute* 41(2):351-357

Medawar (1958): Part played by deoxyribonucleic acid in transplantation immunity. *Nature* 182:62

Meek ES, Hewer TF (1959): An intestinal carcinoma in mice following injeciton of herring-sperm deoxyribonucleic acid. *Brit J Cancer* 13:121-125

Mercola KE, Stang HD, Browne J, Salser W, Cline MJ (1980): Insertion of a new gene of viral origin into bone marrow cells of mice. *Science* 208:1033-1035

Miller A, Rosman G (1989): Improved retoviral vectors for gene transfer and expression. *BioTechniques* 7(9):980-990

Miller AD (1992): Human gene therapy comes of age. *Nature* 357:455-60

Miller AD, Law M-F, Verma EM (1985): Generation of helper-free amphotropic retroviruses that transduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene. *Molecular and Cellular Biology* 5(3):431-437

Minson AC, Wildy P, Buchan A, Darby G (1978): Introduction of the Herpes simplex virus thymidine kinase gene into mouse cells using virus DNA or transformed cell DNA. *Cell* 13:581-587

Morrow JF (1976): The prospects for gene therapy in humans. *Annals of the New York Academy of Sciences* 265:13-21

Mountain IM, Alexander HE (1959): Infectivity of ribonucleic acid (RNA) from type I poliovirus in embryonated egg. *Proc Soc Exp Biol Med* 101:527-532

Mulligan RC, Berg P (1980): Expression of a bacterial Gene in mammalian Cells. *Science* 209:1422-1427

Mulligan RC, Berg P (1981): Selection for animal cells that express the Escherichia

coli gene coding for xanthine-guanine phosphoribosyltransferase. *Proc Natl Acad Sci USA* 78:2072-2076
igan RC, Berg R (1981): Factors Governing the Expression of a bacterial Gene in Mammalian Cells. *Molecular and Cellular Biology* 1(5):449-459
igan RC, Howard B, Berg P (1979): Synthesis of rabbit beta-globin in cultured monkey kidney cells following infection with a SV40 beta-globin recombinant genome. *Nature* 277:108-114
yon W, Kraiselburd E, Davis D, Mann J (1971): Transfer of thymidine kinase to thymidine kinaseless L cells by infection with Unltraviolet-irradiated herpes simplex virus. *Journal of Virology* 7(6):813-820
lle R (1976): Gene therapy and the ethics of genetic therapeutics. *Annals of the New York Academy of Sciences* 265:153-169
lau C, Le Pape A, Soriano P, Fargette F, Juhel M-F (1983): In vivo expression of rat insulin after intravenous administration of the liposome-entrapped gene for rat insulin I. *Proc Natl Acad Sci USA* 80:1068-1072
lson MO, McAllister RM (1972): Infectivity of Human Adenovirus-1 DNA. *Virology* 48:14-21
G, Vielle F, Changeux JP (1967): On the arginase of the shope papillomas. *Virology* 31:729-32
hkis KE, Cantarow A, Stansney J (1955): Induction of neoplasms by injection of tumor chromatin. *Journal of the National Cancer Institute* 15(5):1525-1532
cer A, Robins D, Wold B, Sweet R, Jackson J, Lowy I, Roberts JM, Sim GK, Silverstien S, Axel R (1980): Altering genotype and phenotype by DNA-mediated gene transfer. *Science* 209:1414-1422
y TL, Walker D (1958): Failure of deoxyribonucleic acid to effect somatic transformation in the rat. *Proc Soc Exp Biol Med* 99:717-720
tz M (1992): *Protein Structure: New approaches to disease and therapy*. New York: W.H. Freeman and Co.
pson L, Lonberg-Holm K, Pettersson U (1968): Virus-receptor interaction in an adenovirus system. *Journal of Virology* 2(10):1064-1075
ication N (1971). The prospects of Gene Therapy. In E. Freese (Ed.), *Fogarty International Center*, Bethesda, Maryland: DHEW Publication
otti GF (1963): Incorporation of DNA into a mouse tumor in vivo and in vitro. *Experimental Cell Research* 31:562-565
naud A, Cloastre L, Bernard J, Laveran H, Ackermann H-W, Licois D, Joly B (1992): Characteristics and diffusion in the rabbit of a phage for escherichia coli 0103. Attempts to use this phage for therapy. *Vet Microbiol* 30:203-212
te WO (1962): The in vivo reutilization of lymphocytic and sarcoma DNA by cells growing in the peritoneal cavity. *J Cell Biol* 13:205-216
ers S (1959): Induction of arginase in rabbit epithelium by the Shope rabbit papilloma virus. *Nature* 183:1815-1816
ers S, Lowenthal A, Terheggen HG, Columbo JP (1973): Induction of arginase activity with the shope papilloma virus in tissue culture cells from an argininemic patient. *Journal of Experimental Medicine* 137:1091-1096

Rogers S, Moore M (1963): Studies of the mechanism of action of the shope rabbit papilloma virus. I. Concerning the nature of the induction of arginase in the infected cells. *J Exp Med* 117:521-542
Rogers S, Pfuderer P (1968): Use of viruses as carriers of added genetic information. *Nature* 219:749-751
Rothberg S, Van Scott EJ (1961): Localization of Arginase in Rabbit Skin. *Nature* 189:832-833
Sambrook J, Westphal H, Srinivasan PR, Dulbecco R (1968): The integrated state of viral DNA in SV40-transformed cells. *Proc Natl Acad Sci USA* 60:1288-1295
Scangos G, Ruddle FH (1981): Mechanisms and applications of DNA-mediated gene transfer in mammalian cells-a review. *Gene* 14:1-10
Schimizu T, Koyama S, Iwafuchi M (1962): Nuclear uptake of deoxyribonucleic acid by Ehrlich ascites-tunor cells. *Biochim Biophys Acta* 55:795-798
Seegmiller JE, Rosenbloom FM, Kelley WN (1967): Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. *Science* 155:1682-1685
Shih C, Shilo B-Z, Goldfarb MP, Dannenberg A, Weinberg RA (1979): Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. *Proc Natl Acad Sci USA* 76(11):5714-5718
Shimotohno K, Temin HM (1981): Formation of infections progeny virus after insertion of herpes simplex thymidine kinsase gene into DNA of an Avian Retrovirus. *Cell* 26:67-77
Sinsheimer RL (1969): The prospect for designed genetic change. *American Scientist* 57(1):134-142
Sirotnak FM, Hutchison DJ (1959): Absorption of deoxyribonucleic acid by mouse lymphoma cells. *Biochim Biophys Acta* 36:246-248
Smull CE, Ludwig EH (1962): Enhancement of the plaque-forming capacity of poliovirus ribonucleic acid with basic proteins. *J Bacteriol* 84:1035-1040
Soothill JS (1992): Treatment of experimental infections of mice with bacteriophages. *J Med Microbiol* 37:258-261
Spizizen J, Reilly BE, Evans AH (1966): Microbial transformation and transfection. *Ann Rev Microbiol*: 20:371-400
Stacey DW (1980): Expression of a subgenomic retroviral messenger RNA. *Cell* 21:811-820
Stasney J, Cantarow A, Paschkis KE (1950): Production of neoplasms by injection of fractions of mammalian neoplasms. *Cancer Research* 10:775-782
Stolk A (1960): Experimental carcinoma of the intestine in the Cichlid Aequidens maroni (Steindachner) following injection of herring-sperm deoxyribonucleic acid. *Naturwissenschafften* 47:88-89
Szybalska EH, Szybalski W (1962): Genetics of human cell lines, IV. DNA-mediated heritable transformation of a biochemical trait. *Proc Natl Acad Sci USA* 48:2026-2034
Szybalski W (1963). DNA-mediated Genetic Transformation of Human Cell Lines.

In *Round Table Discussion, Poultry Breeders of America Annuel Meeting*, McArdle Memorial Laboratory, UW-Madison:

Szybalski W (1991): A forerunner of monoclonal antibodies and human gene therapy. *Current Contents* 34:11

Szybalski W (1992): Use of the HPRT gene and the HAT selection Technique in DNA-mediated transformation of mammalian cells: first steps toward developing hybridoma techniques and gene therapy. *BioEssays* 14(7):495-500

Tabin CJ, Hoffmann JW, Goff SP, Weinberg RA (1982): Adaptation of a retrovirus as a eucaryotic vector transmitting the herpes simplex virus thymidine kinase gene. *Molecular and Cellular Biology* 2(4):426-436

Tatum EL (1966): Molecular biology, nucleic acids and the future of medicine. *Perspectives in Biology and Medicine* 10:19-32

Temin H (1976): The DNA provirus hypothesis. *Science* 192:1075-1080

Temin HM (1971): Mechanism of cell transformation by RNA tumor Viruses. *Annual Review of Microbiology* 25:609-648

Temin HM, Mizutani S (1970): RNA-dependent DNA Polymerase in Virions of rous sarcoma virus. *Nature* 226:1211-1213

Terheggen HG, Lowenthal A, Lavinha F, Colombo JP, Rogers S (1975): Unsuccessful Trial of gene Replacement in arginase deficiency. *The Journal of Experimental Medicine* 119:1-3

Topp WC, Lane D, Pollack R (1981). Transformation by SV40 and polyoma virus. In J. Tooze (Eds.), *DNA Tumor Viruses* (pp. 205-296). Cold Spring Harbor

Vaheri A, Pagano JS (1965): Infectious Poliovirus RNA: a sensitive method of assay. *Science* 175:434-436

Wade N (1980): UCLA gene therapy racked by friendly fire. *Science* 210:509-511

Wade N (1981): Gene therapy cought in more entaglements. *Science* 212:24-25

Wade N (1981): Gene therapy pioneer draws mikadoesque rap. *Science* 212:1253

Warden D, Thorne HV (1968): The infectivity of polyoma virus DNa for mouse embryo cells in the presence of Diethylaminoethyl-dextran. *Journal of General Virology* 3(371-):377

Watson JD, Crick FHC (1954): Genetical implications of the structure of deoxyribonucleic acid. *Nature* 171:737-739

Wei C-M, Gibson M, Spear PG, Scolnick EM (1981): Construction and isolation of a transmissible retrovirus containing the src Gene of harvey murine sarcoma virus and the thymidine kinase gene of herpes simplex virus type I. *Journal of Virology* 39(3):935-944

Weil R (1961): A quantitative assay for a subviral infective agent related to polyoma virus. *Virology* 14:46-53

Weisberger AS (1962): Induction of altered globin synthesis in human immature erthrocytes incubated with ribonucleoprotein. *Proc Natl Acad Sci USA* 48:68-80

Wigler M, Pellicer A, Silverstein S, Axel R (1977): Transfer of purified Herpes virus thymidine kinase gene to cultured mouse cells. *Cell* 11:223-232

Wigler M, Pellicer A, Silverstein S, Axel R (1978): Biochemical Transfer of Single-Copy Eucaryotic Genes Using Total Cellular DNA as Donor. *Cell* 14:725-731

Wigler M, Pellicer A, Silverstein S, Axel R, Urlaub G, Chasin L (1979): DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. *Proc Natl Acad Sci USA* 76:1373-1376

Willecke K, Klomfa BM, Mierau R, Dohmer J (1979): Intraspecies transfer via total cellular DNA of the Gene for Hypoxanthine phosphoribosyltransferase into cultured mouse cells. *Molecular and General Genetics* 170:179-185

Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, Felgner PL (1990): Direct gene transfer into mouse muscle in vivo. *Science* 247:1465-1468

Zinder ND, Lederberg J (1952): Genetic exchange in Salmonella. *J Bacteriology* 64:679-699